NASA TECHNICAL TRANSLATION NASA TT F-15,189

GOLGI APPARATUS AND ORIGIN OF THE SECRETORY GRANULES IN ADENOHYPOPHYSEAL CELLS IN THE RAT. AUTORADIOGRAPHIC STUDIES WITH THE ELECTRON MICROSCOPE AFTER INJECTION OF TRITIATED LEUCINE

J. Racadot, L. Olivier, E. Porcile and B. Droz
Presented by R. Courrier

Translation of:
"Appareil de Golgi et origine des grains de sécrétion dans les cellules adénohypophysaires chez le Rat. Étude radioautographique en microscopie électronique après injection de leucine tritiée," C.R. Acad. Sc. Paris, Vol. 261, Group 12, October 11, 1965, pp. 2972-2974.

NASA-TT-F-15189) GOLGI APPARATUS AND ORIGIN OF THE SECRETORY GRANULES IN ADENOHYPOPHYSEAL CELLS IN THE CELLS IN THE RAT. (Scientific Translation Service) 13 p HC $3.00 CSCL 06C G3/04 N74-11890 Unclas 23499

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 NOVEMBER 1973

GOLGI APPARATUS AND ORIGIN OF THE SECRETORY GRANULES IN ADENOHYPOPHYSEAL CELLS IN THE RAT. AUTORADIOGRAPHIC STUDIES WITH THE ELECTRON MICROSCOPE AFTER INJECTION OF TRITIATED LEUCINE [*]

Jean Racadot, Léon Olivier, Evelyne Porcile and Bernard Droz
Presented by Robert Courrier

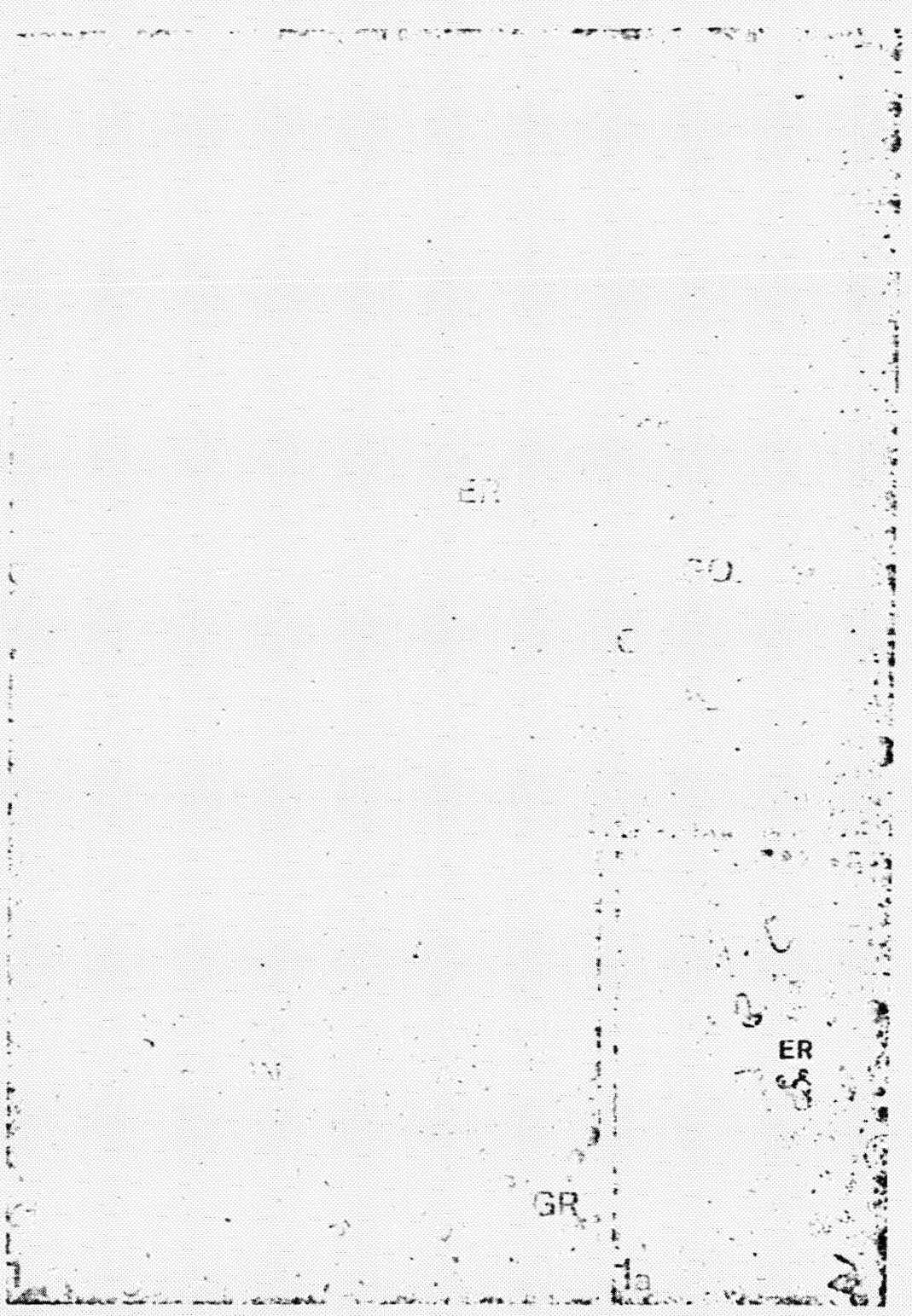
The Golgi apparatus has long been considered as originating /2972* from the secretory granules of adenohypophyseal cells as seen in either the optical microscope [2] or the electron microscope [4, 5]. We believed that autoradiography in combination with electron microscopy could provide direct proof. In effect, a labelled amino acid injected into the animal is incorporated into proteins through synthesis rendering them radioactive as well.

It is thus possible to verify whether or not the Golgi apparatus participates in the development of secretory granules starting from the synthesis of new proteins.

Method. Male rats weighing 37 ± 1 g received an intraperitoneal injection of L-leucine-4.5 ^{3}H (specific activity: 5000 mc/mM)**, representing a dosage of 2 mCi per animal.

The adenohypophyses, excised 10, 30, and 90 min. after injection, were fixed in 2% gluteraldehyde, post-fixed in osmium tetroxide and embedded in epon***. The autoradiographs were developed in Ilford's emulsion L4.

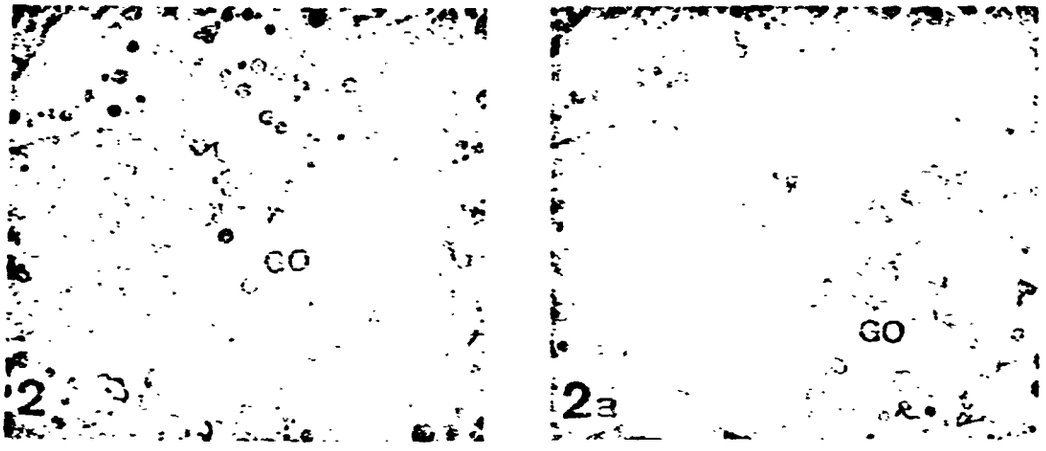
*Numbers in the margin indicate pagination in original foreign text.
**Translator's note: Millicuries/millimole.
***Translator's note: Unknown embedding material.



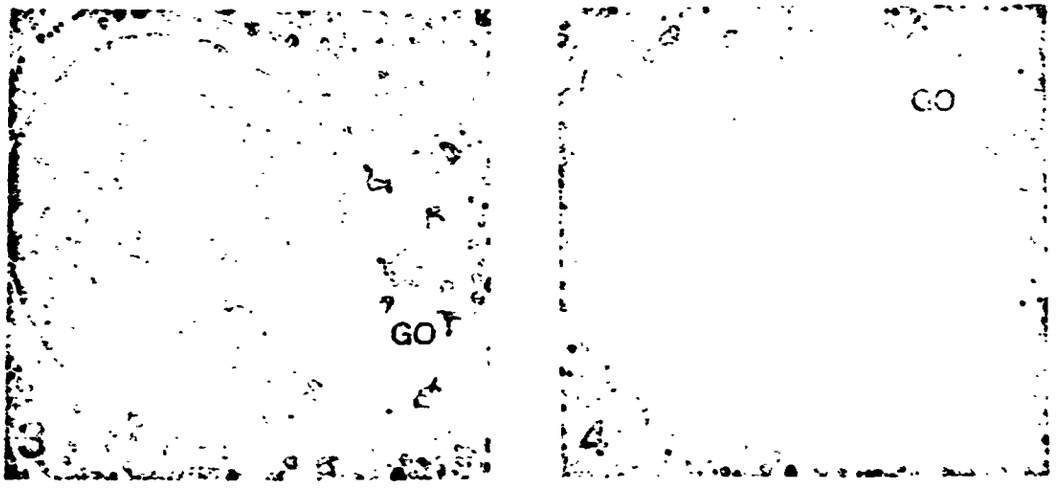
Figures 1 and 1a. (Captions on following page).

Figure 1. (15000 x g) - 10 min. after injection of tritiated leucine the silver particles are situated on the ergastoplasm (ER) and in the perinuclear cisternae (PN). The Golgi apparatus (GO) and the secretory granules (GR) are not labelled. Also note the presence of particles on the chromatin (CH).

Figure 1a. (30,000 x g) - 10 min. after injection, the silver particles are superimposed on the membranes of the ergastoplasmic cisternae (ER) lined with ribosomes.



Figures 2 and 2a. (9000 x g) - 30 min. after injection, the radioactivity accumulates in the Golgi zone in two views of the same cell photographed at 2 different levels. Note the reaction in the intragolgi secretory granules.



Figures 3 and 4. (9000 x g).

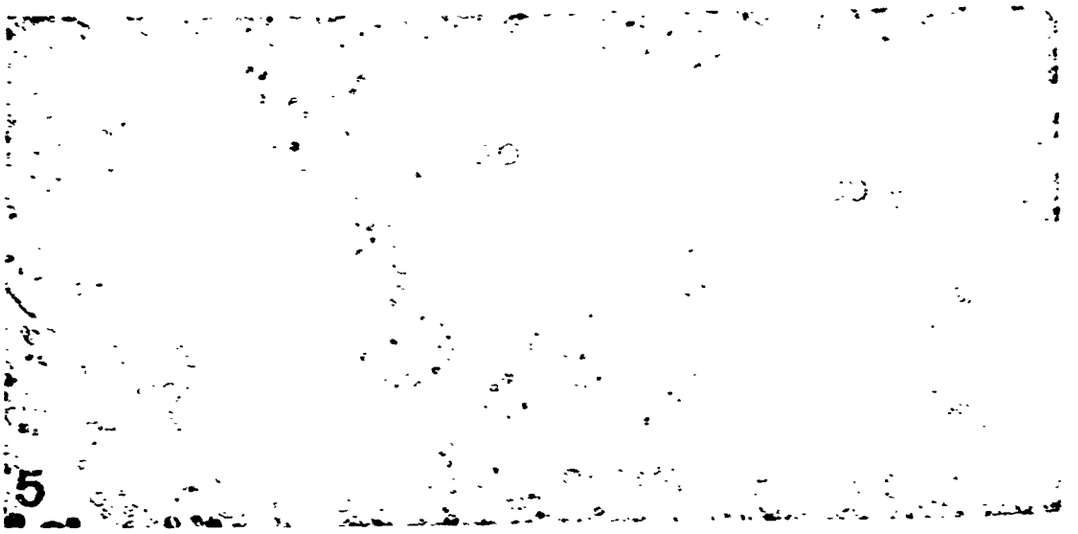


Figure 5. (500 x g) - 30 min. after injection, the silver particles accumulate on the Golgi apparatus especially on the secretory granules in the process of enlarging (white arrow) or completely enlarged.

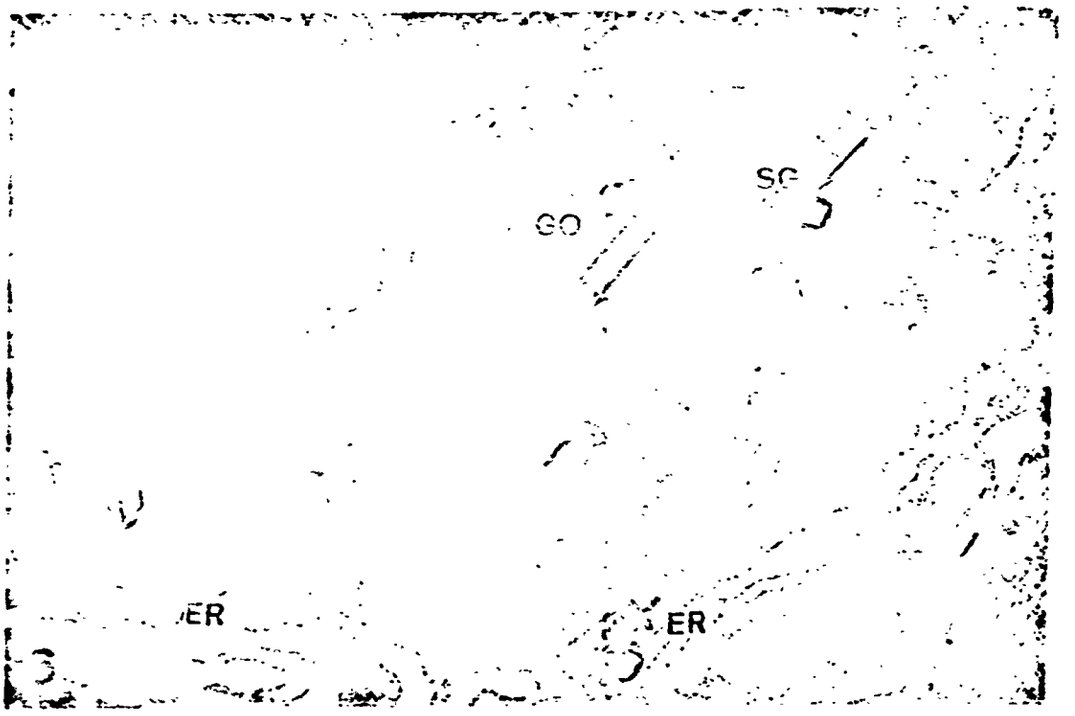


Figure 6. (20,000 x g) - 30 min. after injection the silver particles are found at the same time on the lumen of the ergastoplasm (ER) and on the Golgi saccules (SG). One of these saccules contains a secretory granule still slightly dense (white arrow). Other mature secretory granules are labelled in the Golgi apparatus (‡).

The distribution of autoradiographic activity was systematically studied by serial sections photographed at uniform magnification of 1500 and 3000 with the Siemens Elmiskop I, so as to choose morphologically uniform cells. /2973

Results. Regardless of time sampled, an autoradiographic reaction was evident in the nucleus and mitochondria.

Ten minutes after injection with leucine ^{3}H, most of the silver particles are distributed on the ergastoplasm (Figure 1). As the latter expands, the reaction is localized on the cisternal membranes which carry the ribosomes (Figure 1a). A few silver particles can be occasionally detected on the Golgi apparatus.

At 30 min., the autoradiographic reaction is discrete on the "lumen" of the ergastophasmic cisternae (Figure 6), while it is intense on the Golgi apparatus (Figure 2-7). In the Golgi apparatus, the silver particles are seen at the same time on the vessicles, the saccules (Figure 7), and principally on the secretory granules (Figure 2-7); the latter are occasionally slightly opaque to electrons (Figure 5 and 6, white arrow).

After 90 min., the radioactivity has disappeared from the Golgi apparatus (Figure 8 and 9). It is now localized in a limited number of secretory granules dispersed randomly in the cell. The labelled secretory granules often contain many silver particles (Figure 8).

Discussion. The early reaction observed on the membranes of the ergastoplasmic cisternae (Figure 1-1a) is really due to protein synthesis on the ribosomes [6]. Once synthesized, the proteins effectuate a series of migrations. First, they pass into the lumen of the ergastoplasmic cisternae in a more or less flocculent radioactive form (Figure 6) and from there, reach the vessicles

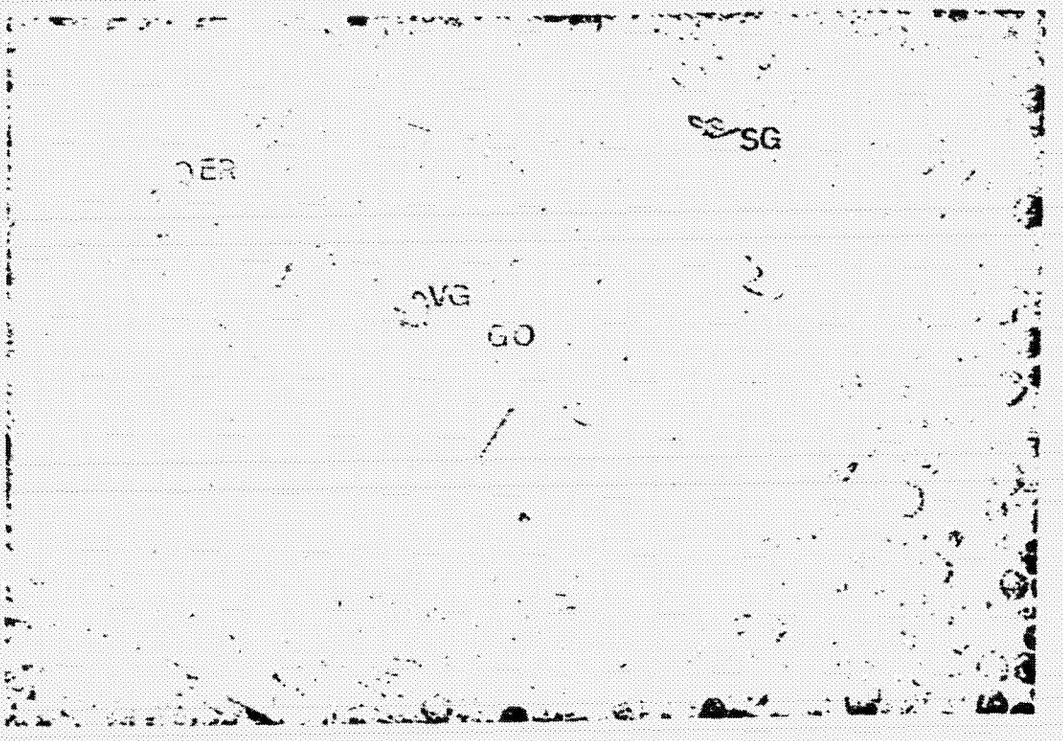


Figure 7. (20,000 x g) - 30 min. after injection, the radioactivity is localized at the level of the ergastoplasmic cisternae (ER), the saccules (SG) and the Golgi vessicles (VG).

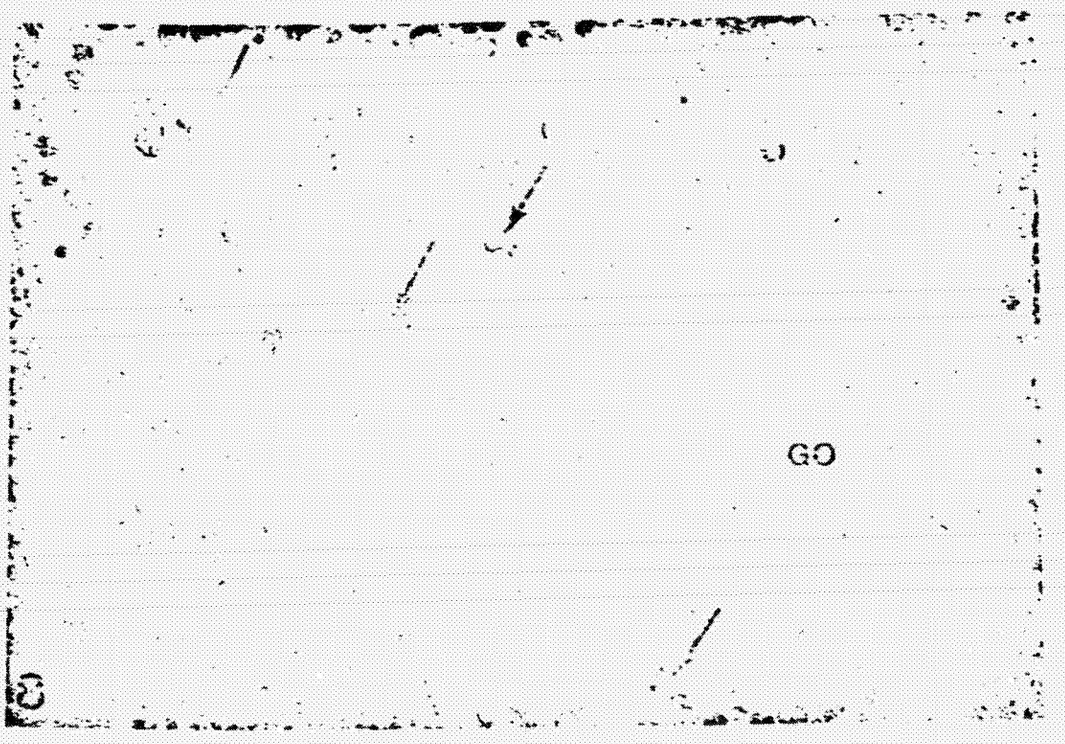


Figure 8. (15,000 x g) - 90 min. after injection, the Golgi apparatus (GO) is no longer labelled. The autoradiographic reaction is localized on the secretory granules dispersed in the cytoplasm.

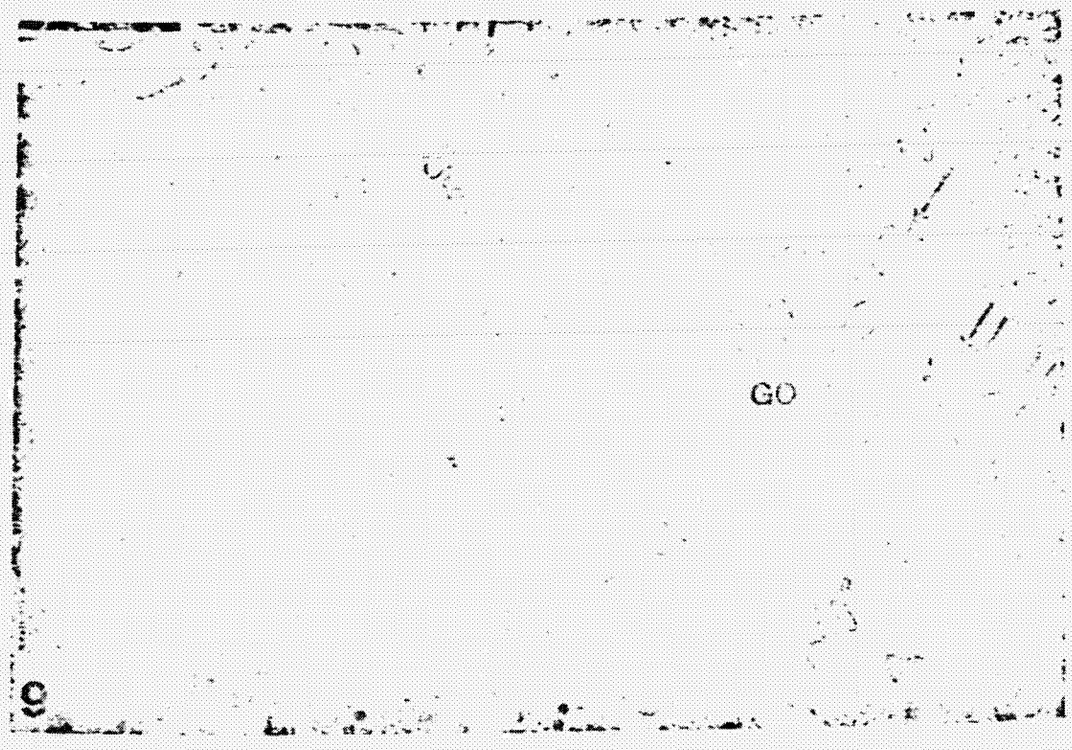


Figure 9. (15,000 x g) - 90 min. after injection all radioactivity has disappeared from the Golgi apparatus (GO). The labelled secretory granules in the peripheral cytoplasm are strongly radioactive. Two or 3 silver particles can be counted at their positions (‡).

and saccules of the Golgi apparatus [7] (Figures 6 and 7). Since the Golgi apparatus is already labelled lightly after 10 min., we can deduce that protein transport must rapidly proceed from the ergastoplasm in the Golgi apparatus. These findings have already been made in the pancreas [9].

The newly formed proteins accumulate in certain saccules expanded to a form which becomes rapidly opaque to electrons (Figures 5 and 6, white arrow). At this stage the protein material surrounded by the vacuolar membrane seem to condense and to progressively acquire the appearance of the secretory granules (Figures 2 and 7).

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Thus, as in the pancreatic exocrine [1, 8], the earliest labelled secretory granules originate in the bosom of the Golgi zone between the tenth and thirtieth min., and most likely in less than 20 min.

Afterwards, the secretory granules which are newly formed leave the Golgi zone (Figures 8 and 9) and disperse throughout the cytoplasm among the pre-existing population of secretory granules. It should be noted that the distribution of silver particles on the secretory granules does not correspond to a random type of distribution: the radioactivity is concentrated in only a small number of secretory granules (Figure 8 and 9). All the evidence indicates that this population encompasses secretory granules of different ages.

We deduced that the secretory granules of adenohypophyseal cells are constructed de novo and in toto in the bosom of the Golgi apparatus, starting from the proteins which were synthesized just beforehand in the ergastoplasm.

REFERENCES

1. Caro, L.G. and G.E. Palade. C. R. Soc. Biol., Vol. 155, 1961, p. 1750.

2. Gatz, A.J. Anat. Rec., Vol. 69, 1937, p. 429.

3. Granboulan, N. and Ph. Granboulan. J. Exptl. Cell. Res., Vol. 38, 1965, p. 604.

4. Haguenau, F. and W. Bernhard. Arch. Anat. Micr., Vol. 44, 1955, p. 27.

5. Herlant, M. Biol. Médicale, Vol. 53, 1964, p. 29.

6. Siekevitz, P. and G.E. Palade. J. Biophysic and Biochem. Cytol., Vol. 7, 1960, p. 619.

7. Tixier-Vidal, A., S. Fiske, R. Picart and F. [illegible]. Comptes rendus, Vol. 261, 1965, p. 1133.

8. Warshawsky, H. and C.P. Leblond, Anat. Rec., Vol. 139, 1961, p. 284.

9. Warshawsky, H., C.P. Leblond and B. Droz. J. Cell. Biol., Vol. 16, 1963, p. 1.

Translated for National Aeronautics and Space Administration under contract No. NASw 2483, by SCITRAN, P.O. Box 5456, Santa Barbara, California, 93108